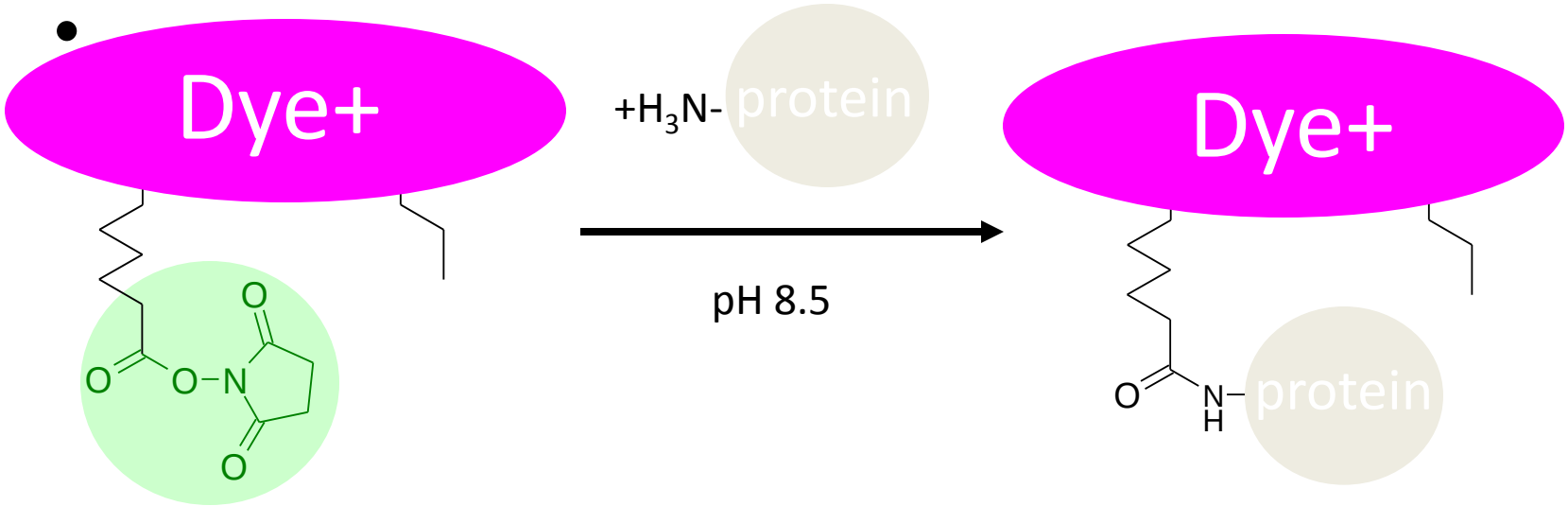
CyDye™ 荧光标记原理

Fluor minimal properties

Labelling protocols

Testing labelling efficiency

Labelling Chemistry

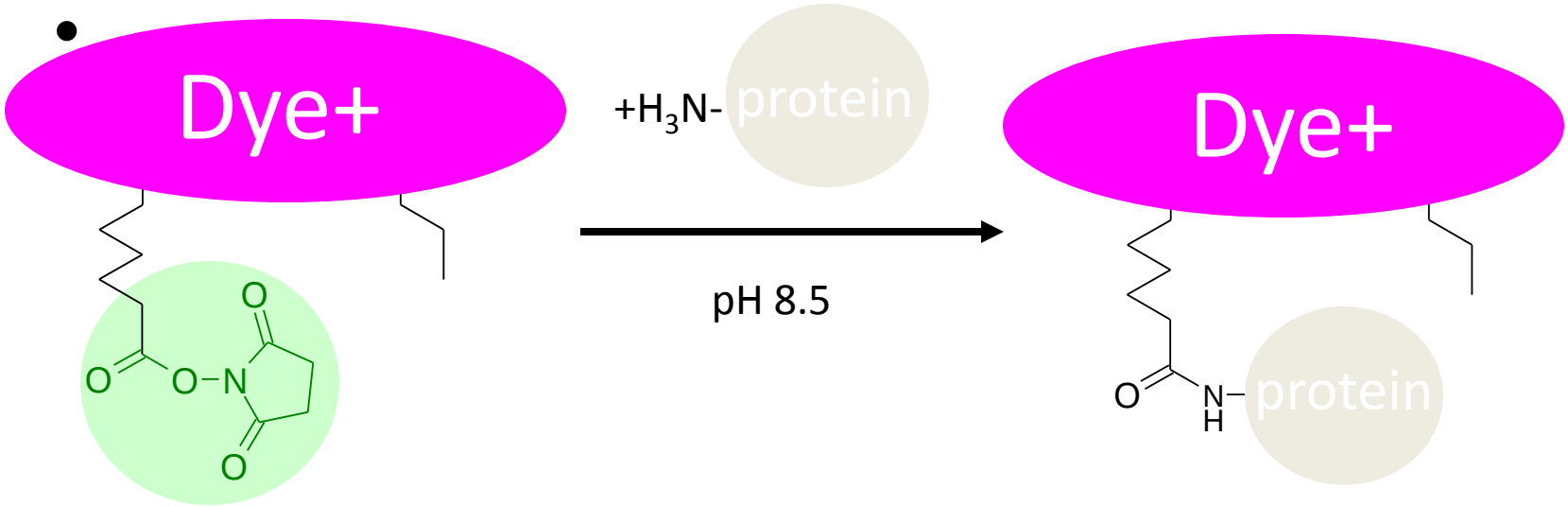


NHS reactive group

Minimal labelling - only 1-2 % of lysines are labelled

Only one spot per protein

Labelling Chemistry



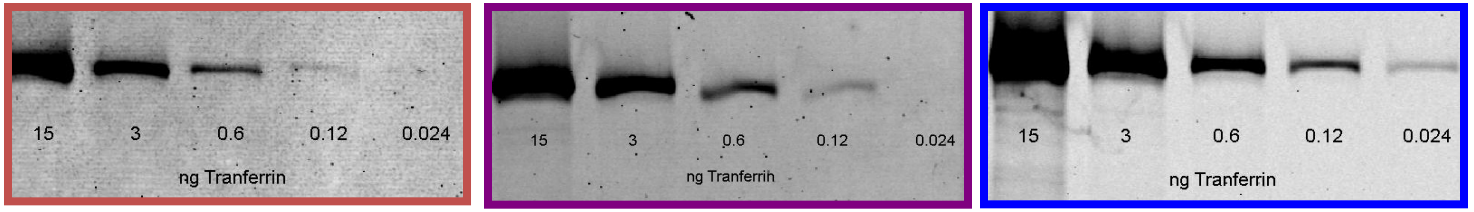
NHS reactive group

Minimal labelling - only 1-2 % of lysines are labelled

Only one spot per protein

Sub-nanogram sensitivity

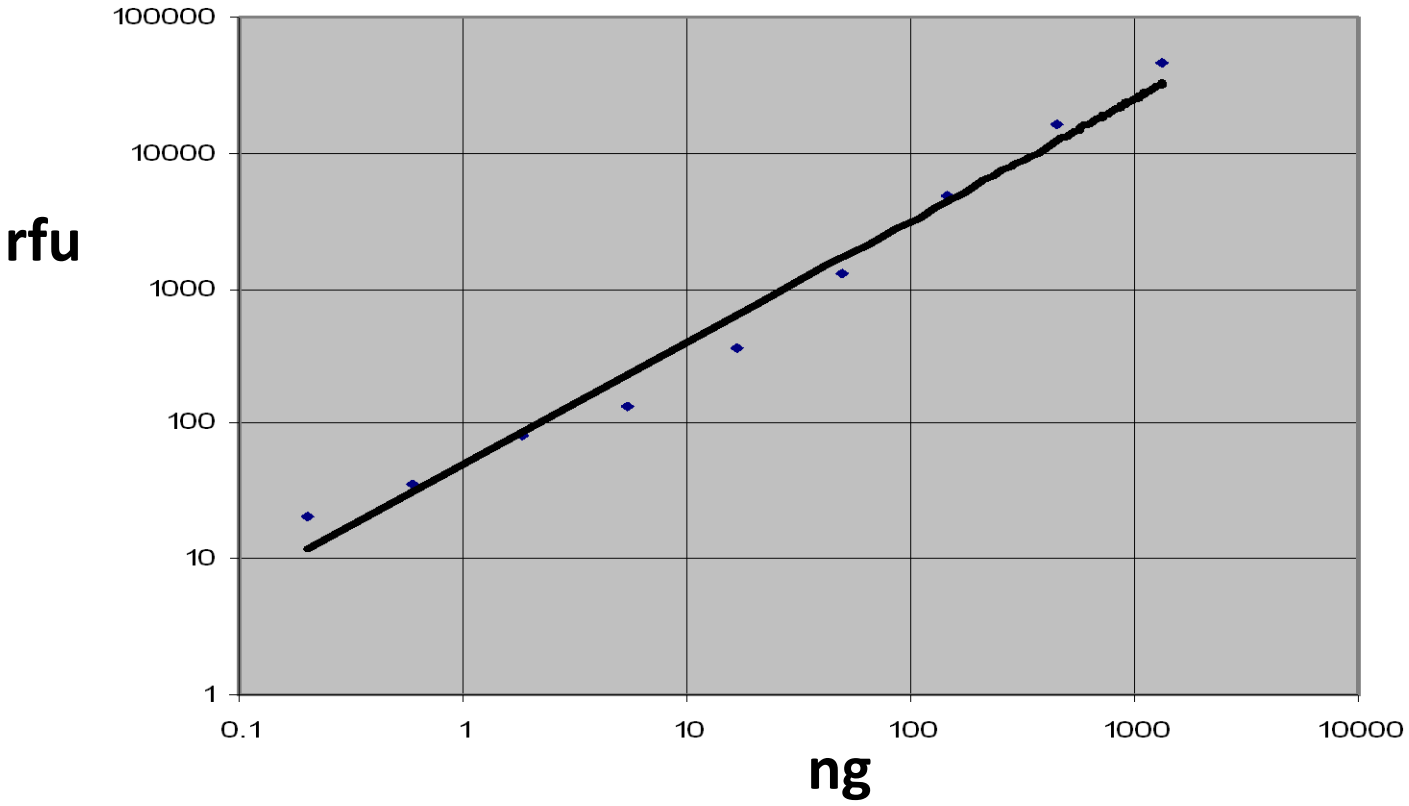
	Cy2	Cy3	Cy5
Ex (nm)	488+/- 0.1	530+/-0.1	633 +/-0.1
Em (nm)	520+/-40	580+/-30	670+/-30
Sensitivity	600 pg	120 pg	24 pg
Max pixel	1074	469	96
Mean bgnd.	718	345	58
Signal:noise	1.5	1.36	1.65



15 3 0.8 0.12 0.024

ng Transferrin

Fluorescent signal is linear across 4 orders of magnitude



Cy5 labelled transferrin separated by 1-D SDS-PAGE

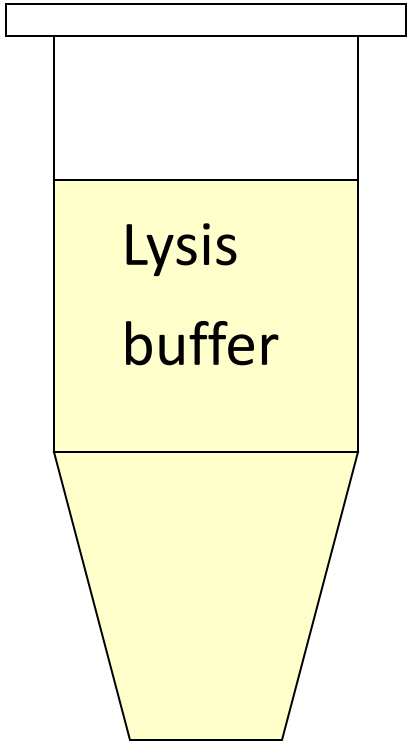
Features and benefits of CyDye™ DIGE Fluor minimal dyes

Size/charge matched	Co-detection of spots on same gel
Net positive charge	No change to pI of protein on labelling No change to the IEF separation profile
Specific minimal labelling	One spot per protein
Spectrally resolvable no cross talk	Distinct signal from each dye
Bright dyes highly sensitive	Enables use of minimal labelling
Photostable	Minimal signal loss during labelling, separation and scanning
pH insensitive	No change in signal over wide pH range used during IEF separation

CyDye™ DIGE Fluor minimal dye properties

Testing labelling efficiency

Step 1 - protein extraction



30 mM Tris

pH 8.5

4 % CHAPS

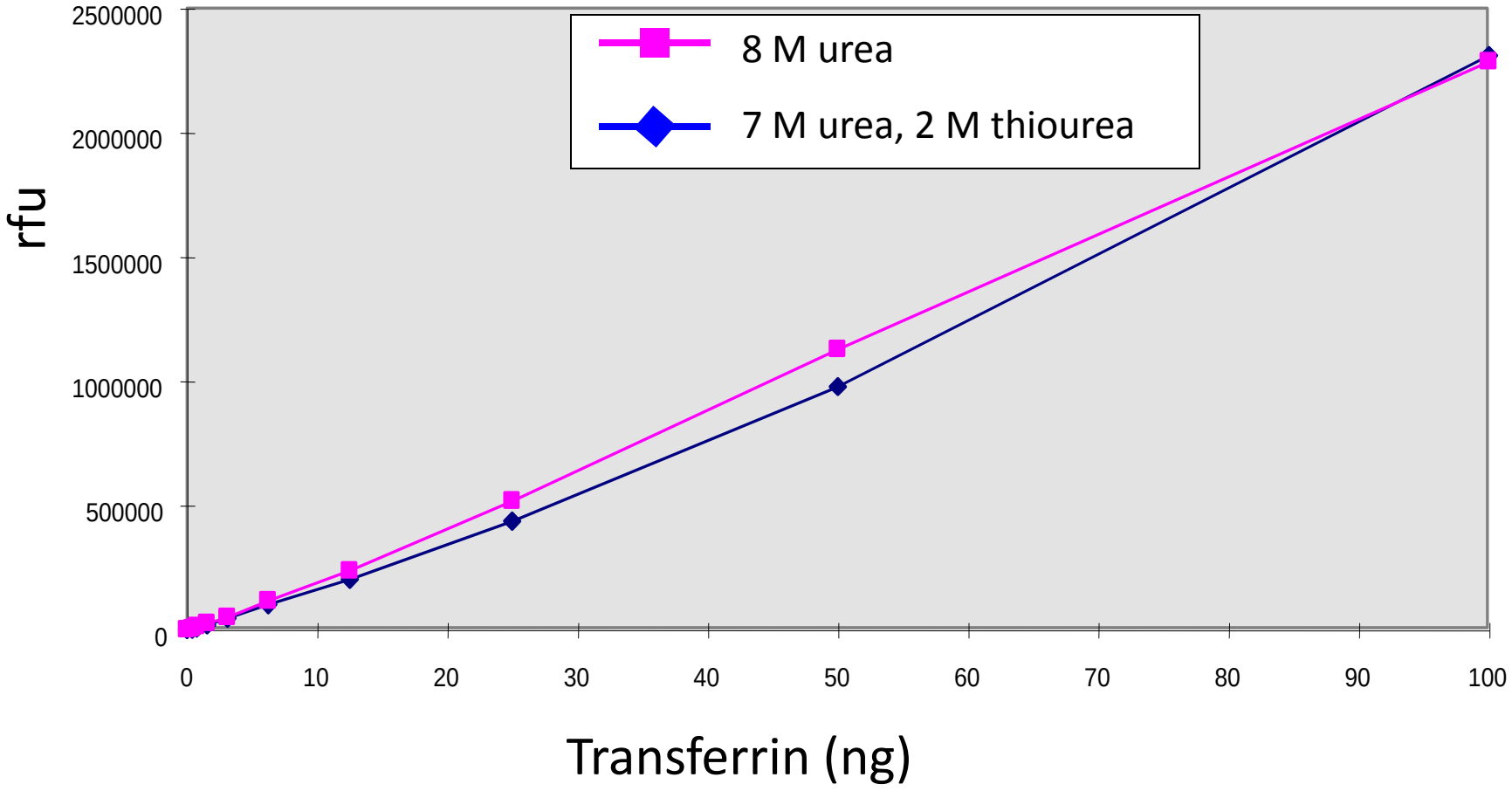
7 M urea

2 M thiourea

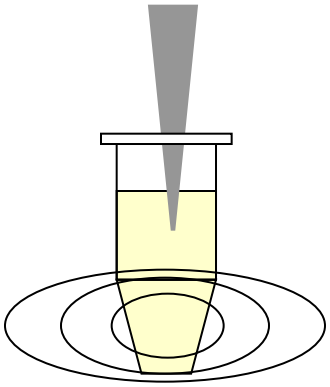
NO Primary amines (e.g. pharmalytes)

NO Thiols (e.g. DTT)

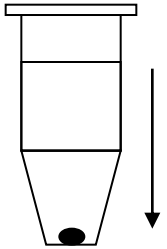
Labelling in presence of urea/thiourea



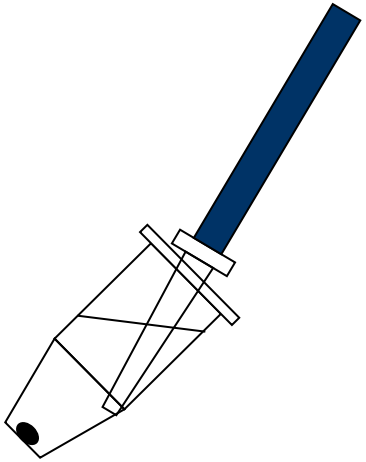
Step 1 - protein extraction



Sonicate



Centrifuge



Extract supernatant

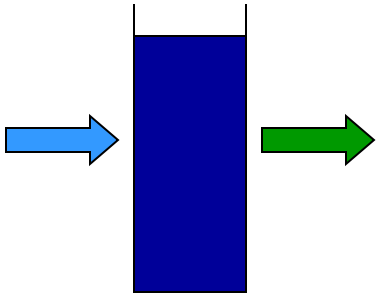
Step 1 - protein extraction

Check the pH is 8.0-9.0

Adjust if necessary using different pH lysis buffer

Quantify (e.g. Ettan™ 2-D Quant Kit)

Protein concentration should be 5-10 mg/ml



Step 2 - Protein labelling

Allow dyes to warm to room temperature before opening

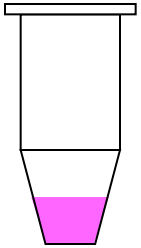
Add 25 µl dry DMF to each tube (1 mM)

Do not use DMF open for more than 3 months

Vortex and spin down

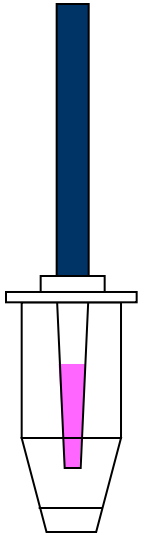
1 mM dyes are stable for 2 months at -20 ºC (dark)

Step 2 - Protein labelling



Prepare the working solution of dye (400 pmoles/ul)

This solution is stable for 1 week at -20 ºC

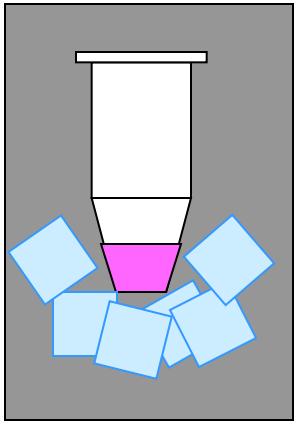


Add the dye to the protein solution

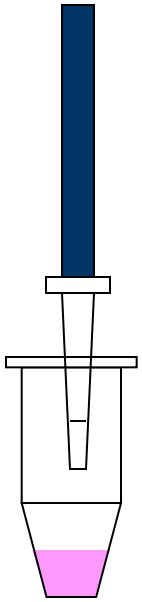
Use 400 pmoles dye per 50 μg protein

Vortex, spin

Incubate on ice for 30 min in the dark



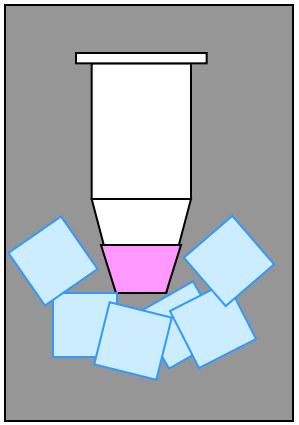
Step 2 - Protein labelling



Quench by adding excess 10 mM lysine

Vortex, spin

Incubate on ice for 15 min



Labelled protein is stable for 3 months at - 70 ºC

Dos and don’ts of minimal labelling

- DO always check the pH of a lysate before labelling
- DO Label between pH 8.0-9.0
- DO include thiourea and urea in lysis buffer
- DON’T Include primary amines or thiols that will compete for the dye
- DO check compatible reagents list (Ettan™ DIGE System User Manual) for other interfering components

When to do a labelling optimisation

A new sample type is being used

Use of a compound not tested for compatibility

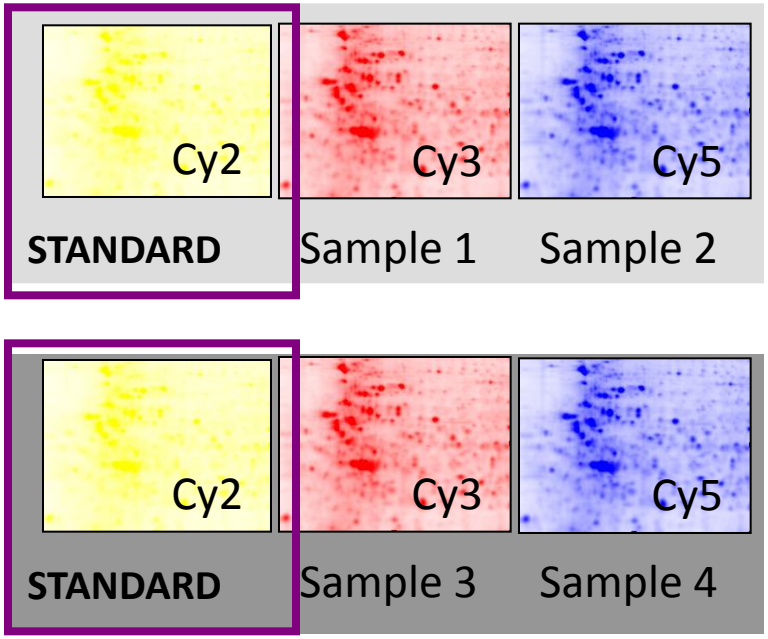
Use of a compound outside the recommended concentration range

Use of a combination of compounds which effect labelling

实验设计

1. Incorporate an internal standard

An internal standard should be include in every gel



This ensures accurate quantitation and aids matching

2. Randomize

Good experimental practice

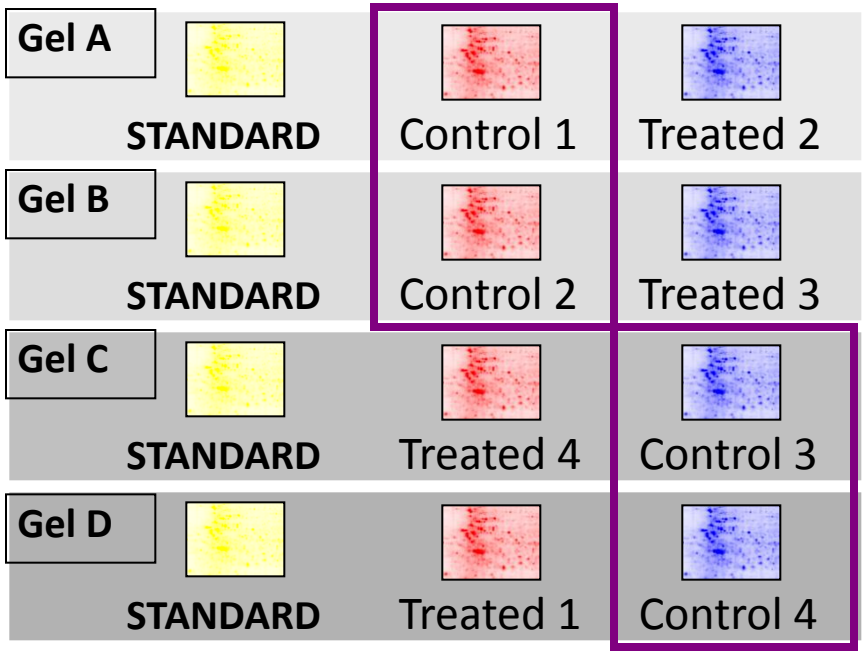
Avoids any bias from

- experimental conditions
- sample handling
- labelling

Peer acceptance of results

2. Randomize - within each group

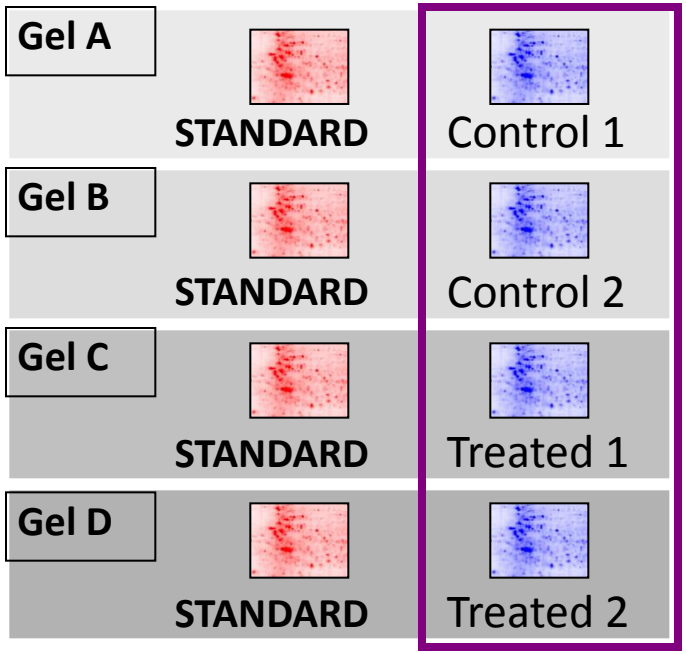
Label half of each group with Cy™3 and half with Cy5



2. Randomize - within each group

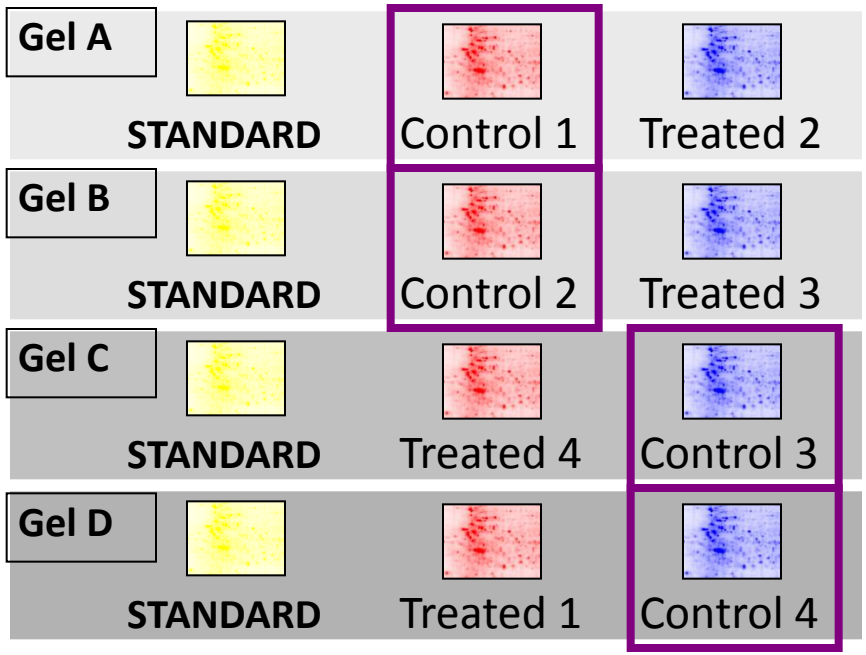
Not required when using 2-colour 2-D DIGE

All groups in the same colour



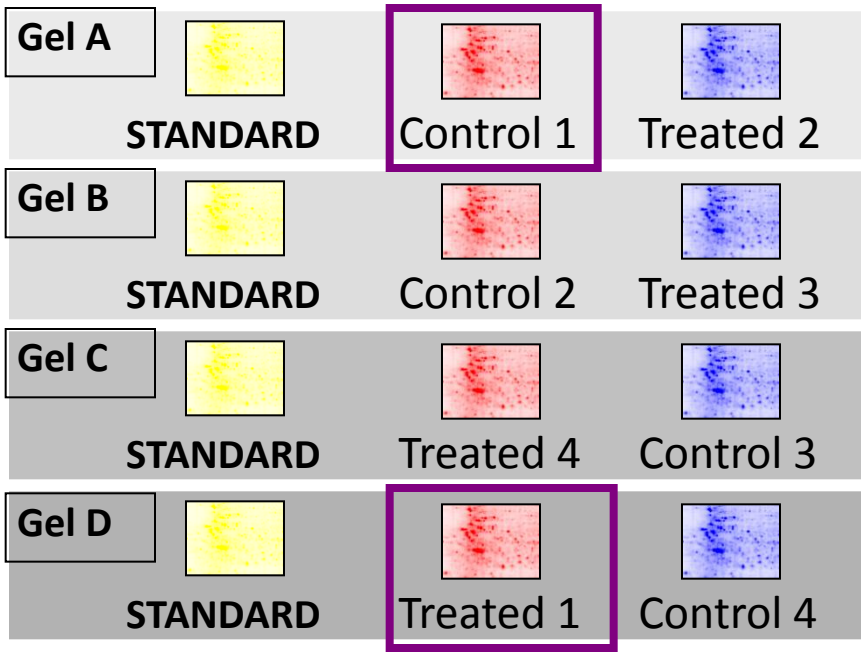
2. Randomize - within each group

Run samples from same group on different gels



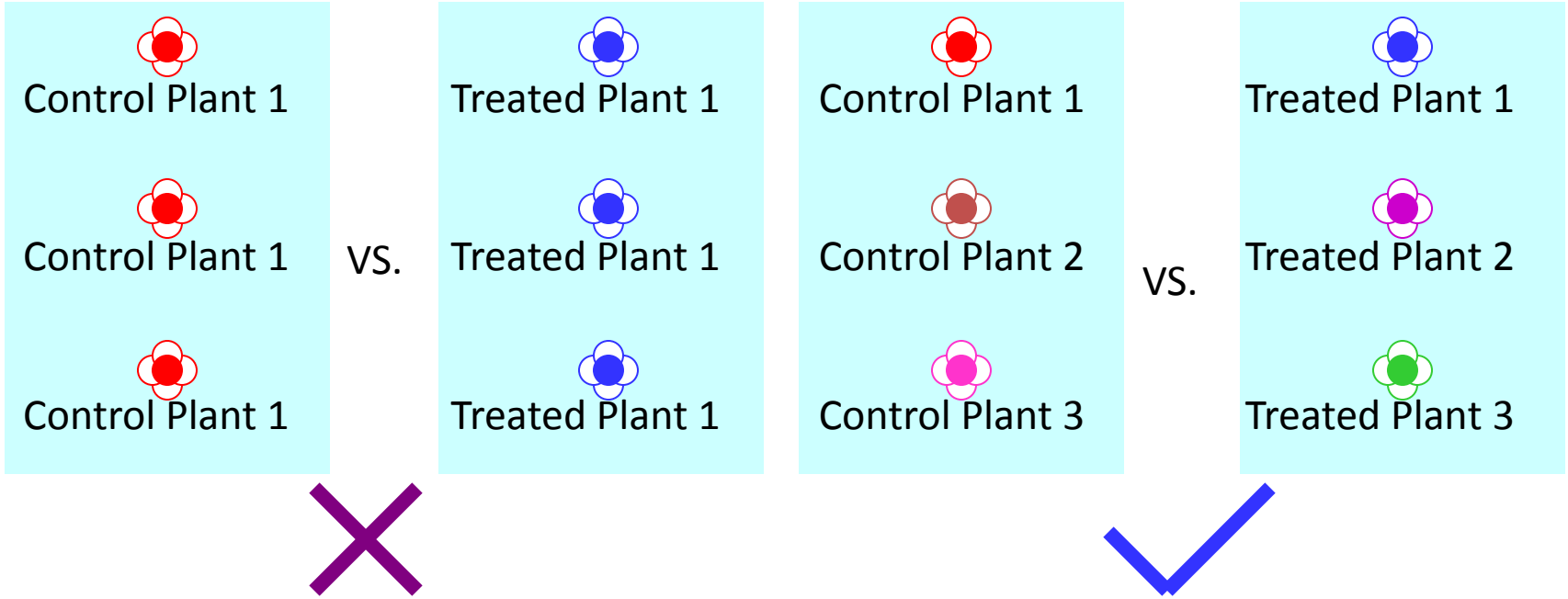
2. Randomize - for each individual

Run samples from same individual on different gels



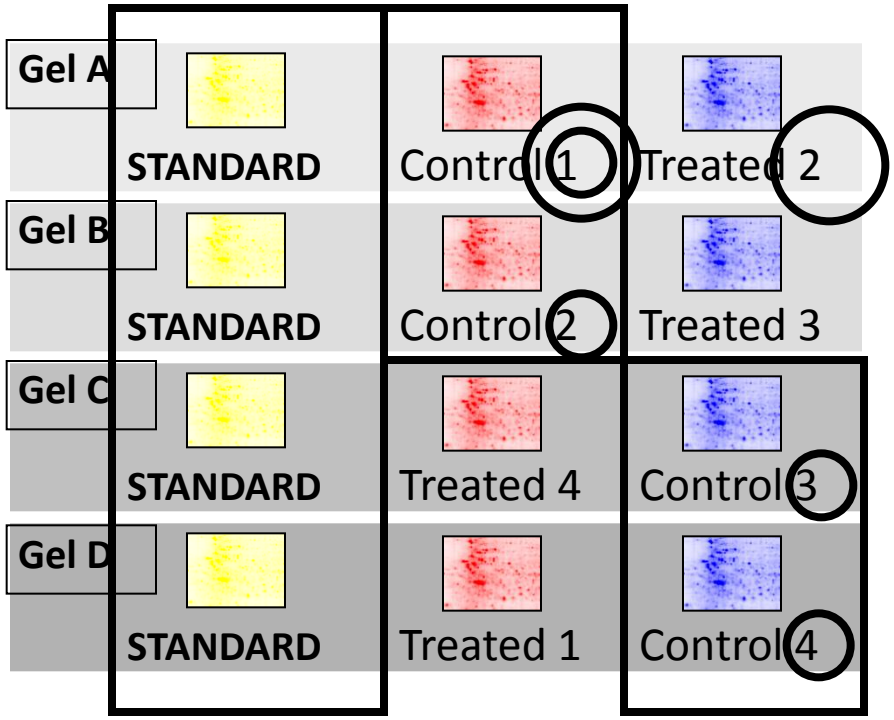
3. Include biological replicates

Use biological replicates instead of gel replicates.



Increases confidence that differences are real induced changes and not inherent biological variation

Correct experimental design



1. Incorporate an internal standard

2. Randomize

3. Include biological replicates